APPROVED BY DRAFTSMAN | O. . F.G. | CLASS | SUBCLASS

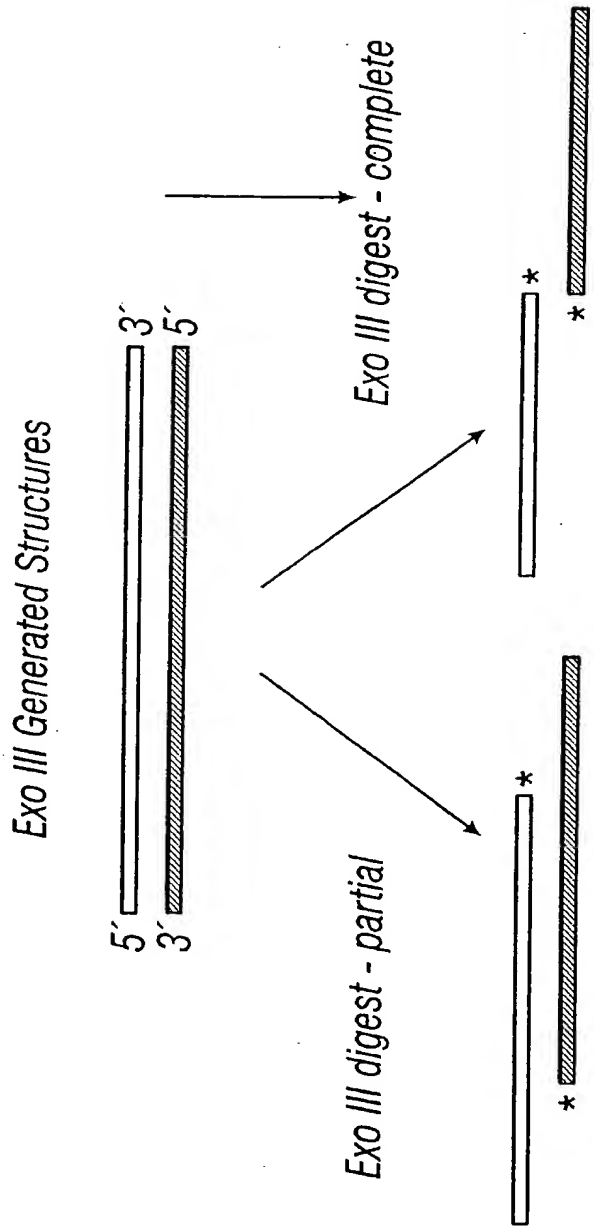


FIG. 1

APPROVED BY DRAFTSMAN | C. . FIG. | CLASS | SUBCLASS

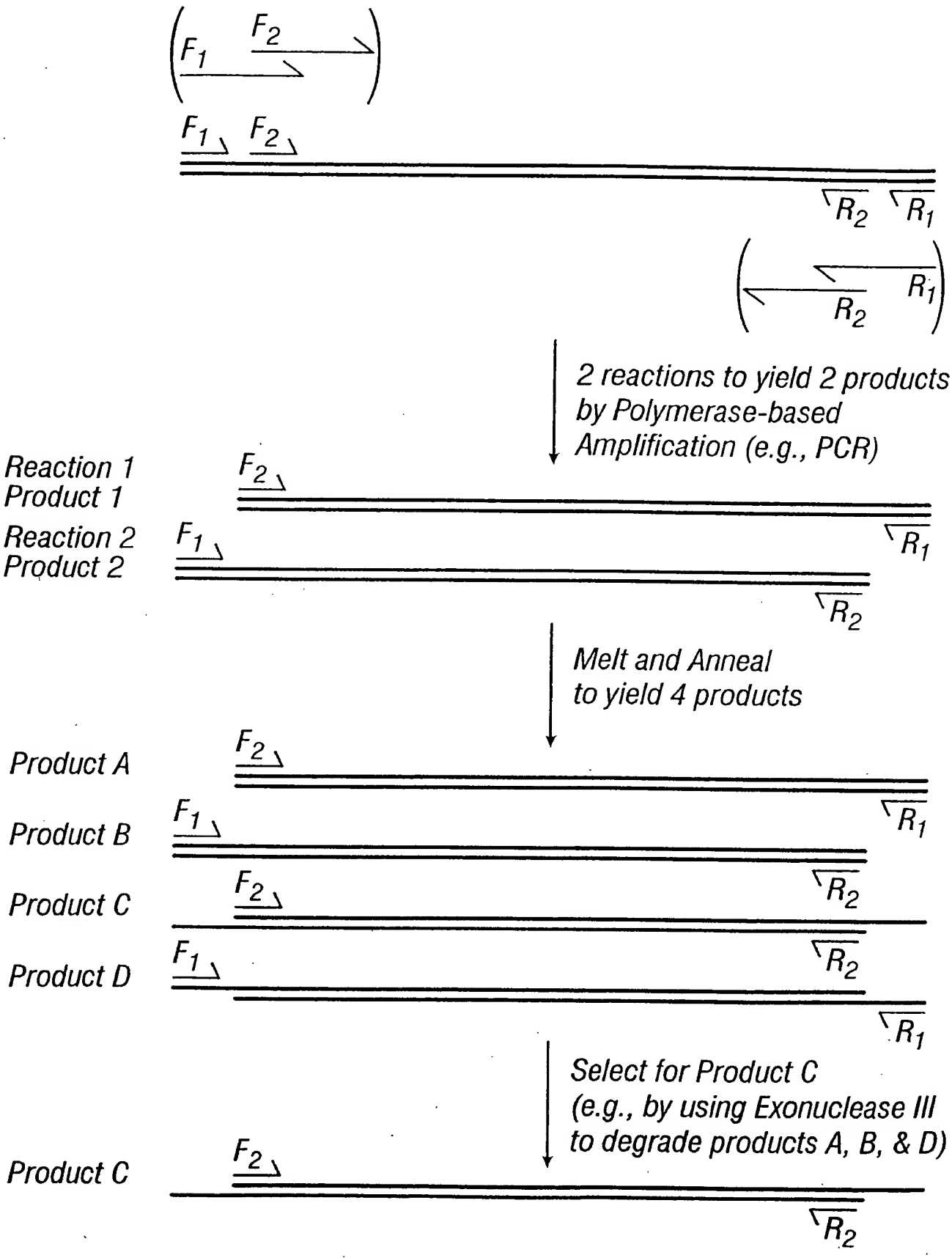


FIG. 2

APPROVED BY DRAFTSMAN | O. . FIG. | CLASS | SUBCLASS

Panel A.

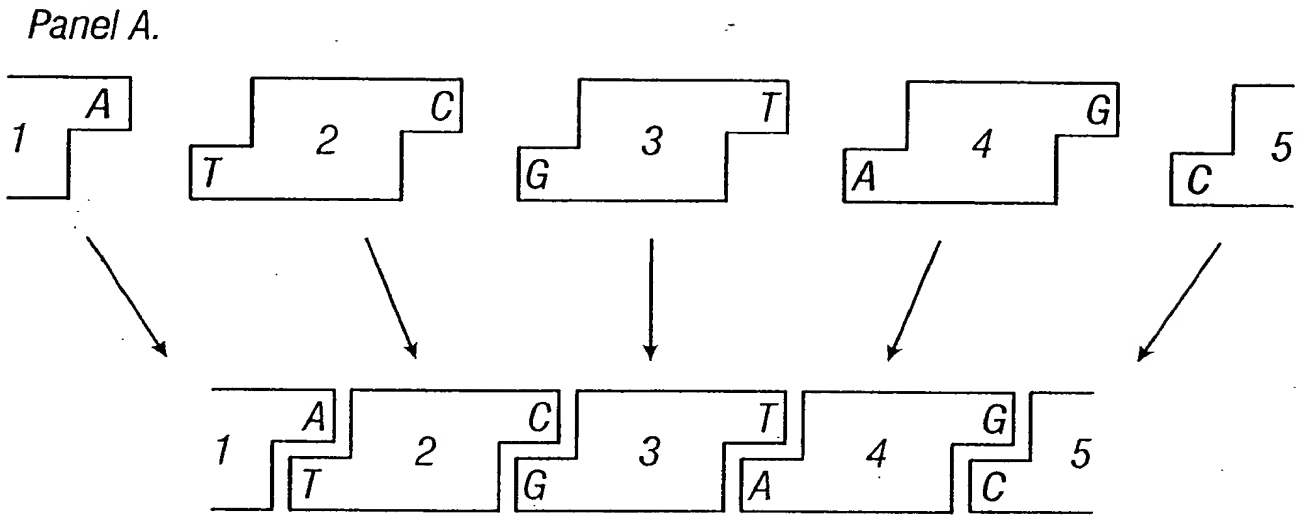
Panel B.

Panel C.

Panel D.

FIG. 3

APPROVED BY DRAFTSMAN | C . FIG. | CLASS | SUBCLASS



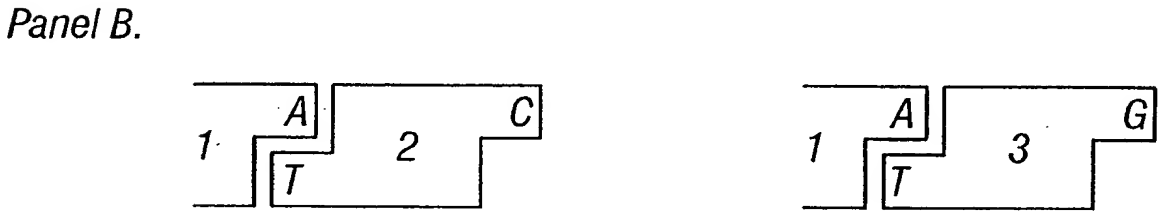


FIG. 4A

APPROVED BY DRAFTSMAN | O. FIG. | CLASS | SUBCLASS

Panel C.

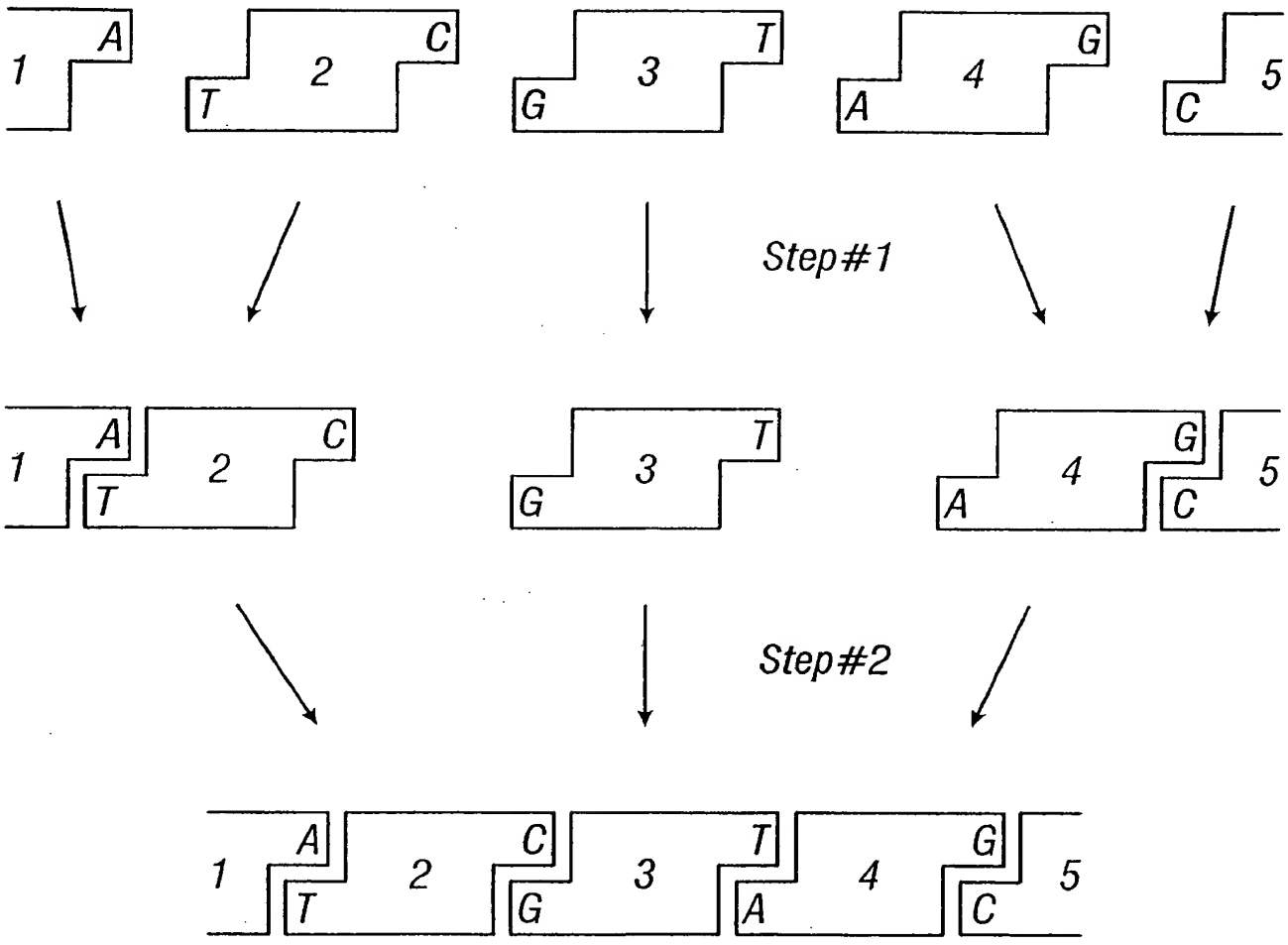


FIG. 4B

APPROVED BY DRAFTSMAN
CLASS
SUBCLASS

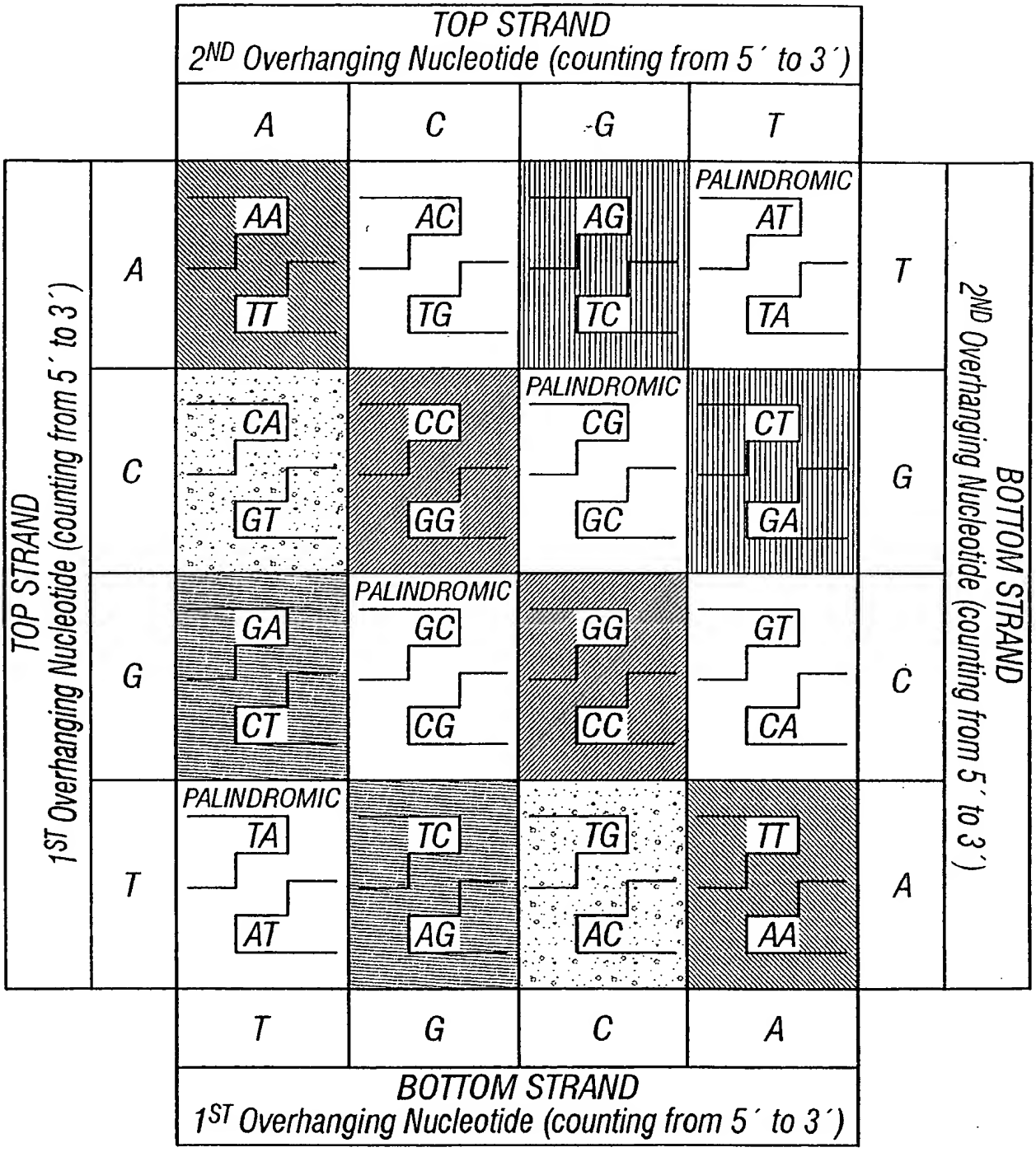


FIG. 5

APPROVED BY DRAFTSMAN CLASS SUBCLASS

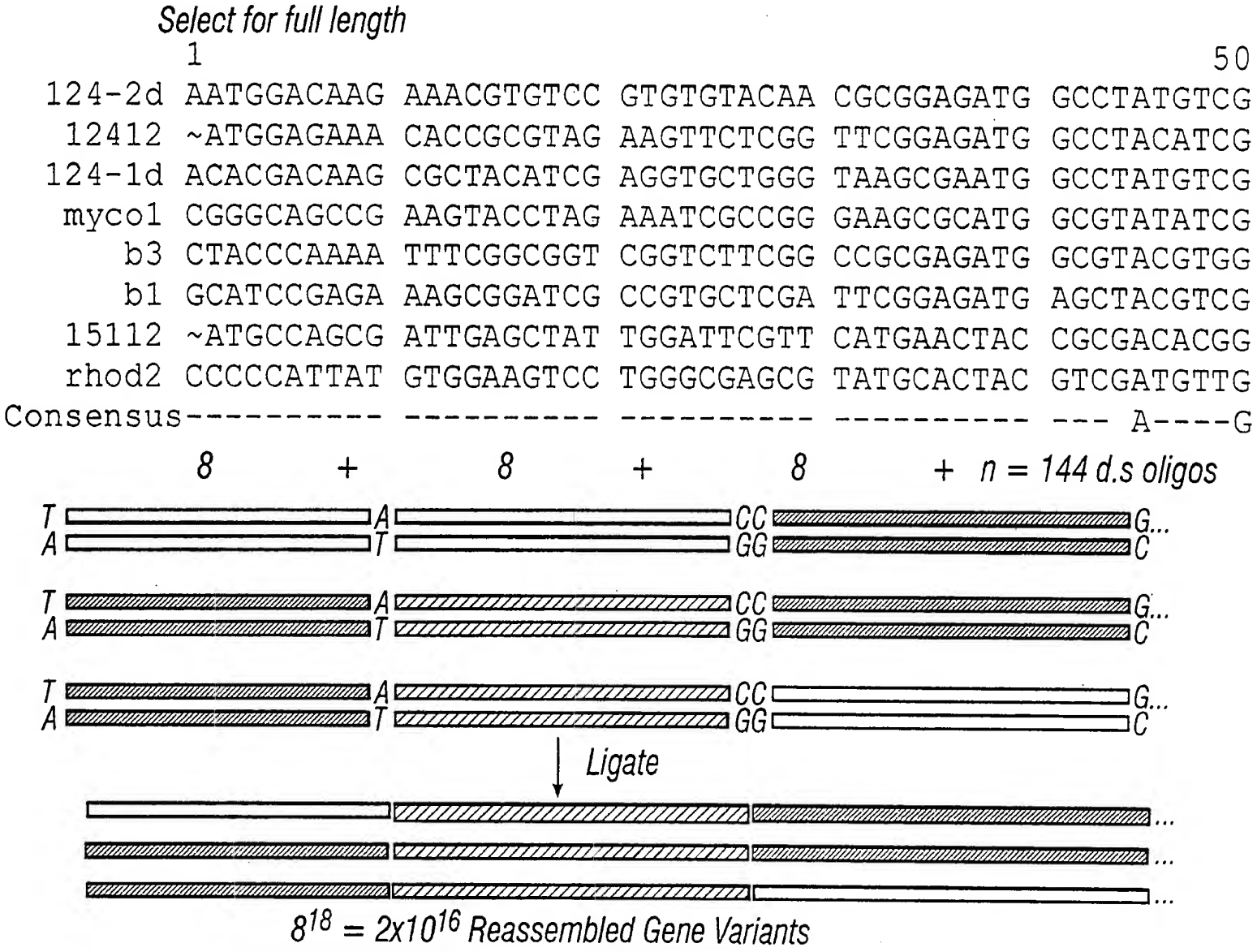


FIG. 6A

```
                                                                    100
ACACGGGCCA GGGTGATTCC GTTCTGTTTC TTCACGGCAA CCCGACGTCG
ACGTGGGAGA GGGCGACCCG ATCGTGTTCC TCCACGGAAA TCCCACGTCG
AGATGGGCGA GGGTGATCCC ATCATTTTCC AACACGGCAA TCCGACCTCA
ACGAAGGCAA GGGTGACGCC ATCGTCTTTC AGCACGGCAA CCCCACGTCG
AAGTGGGACG GGGCGACCCC ATCGTACTCT TGCACGGCAA CCCCACCTCG
ATACCGGCGA GGGAGCGCCG ATCGTGTTCC TTCACGGCAA CCCGACTTCC
GCGTCGGCGA T...CTTCCC GTCGTGTTCC TGCACGGCAA CCCCACGTCG
GACCGCGGGA TGGCACGCCT GTGCTGTTCC TGCACGGTAA CCCGACCTCG
------G--- --------C- -T--T--T-- --CACGG-AA -CC-AC-TC-
```

FIG. 6B

Represents 15% of gene

```
                                                                    150
TCGTATCTGT GGAGGGGCGT AATGCCTTTT GTGACGGACG TCGCCCGATG
TCGTACCTGT GGCGGAACGT GATTCCCCAC GTTGCCGGCT TGGGACGCTG
TCGTACCTGT GGCGCAACAT CATGCCCCAT GTGCAACAGC TCGGTCGCTG
TCTTACTTGT GGCGCAACAT CATGCCGCAC TTGGAAGGGC TGGGCCGGCT
TCGTACCTCT GGCGCAACGT GTTGCCGCAC CTGGCGCCGT TAGGCCGCTG
TCCTATCTTT GGCGCAACAT CATCCCCTAT CTCGCGGATC ACGGCAGATG
TCTCACGTCT GGCGCAACGT GATCCCGCAC GTCGCTGGCC AGCACCGGTG
TCCTACCTGT GGCGCAACAT CATCCCGCAT GTAGCACCGA GTCATCGGTG
TC--A--T-T GG-G---C-T --T-CC---- -T-------- ------G---
```

FIG. 6C

```
                NcoI                                                                CCGT
150am13_00  cATGATGCACG GCGATATTTC ATCGAGCAAT GACACGGTCG GCGTTGCCGT
150AM7_001  cATGCATCACG GCGACATTTC ATCGAGCAAT GACACGGTCG GCGTTGCCGT
431am7_002  cATGAGACACG GAGATATCTC CAGCAGCAAC GATTGCGTGG GCGTGGCCGT

                                                        GAG  GT
150am13_00   CGTGAACTAC AAGATGCCTC GCCTTCATAC CAAGGCGGAG GTTTTAGCGA
150AM7_001   CGTGAACTAC AAGATGCCGC GGCTTCACAC CAAGGCTGAG GTGCTGGCCA
431am7_002   CGTGAACTAC AAGATGCCGC GGCTGCATAC CCGCGCGGAG GTGATGGAGA

                                                             CGG
150am13_00   ACGCCAGAAA GATCGGCGAG ATGATCGTCG GCATGAAGAC CGGCCTGCCC
150AM7_001   ACTGCCGCAA GATCGCCGAC ATGCTGGTCG GCATGAAGAG CGGCCTGCCG
431am7_002   ACGCCCGCAA GATCGCCGAC ATGGTCGTGG GCATGAAGCG CGGCCTGCCC

                                                    CCACG
150am13_00   GGAATGGATC TGGTGATCTT CCCGGAATAT TCGACCCACG GCATCATGTA
150AM7_001   GGAATGGATC TGGTGATCTT CCCGGAATAT TCCACCCACG GCATCATGTA
431am7_002   GGCATGGACC TGGTCATCTT CCCCGAGTAC TCCACCCACG GCATCATGTA

                                                        CCC  GG
150am13_00   CGACTCCAAG GAAATGTACG ATACCGCGTC CGTCGTGCCC GGCGAGGAGA
150AM7_001   CGACTCCAAG GAGATGTACG ACACGGCGTC GACGGTGCCG GGTGAAGAGA
431am7_002   CGACGCCAAG GAAATGTACG AAACCGCTTC GGCCATTCCG GGCGAAGAGA

                                                          G  GGG
150am13_00   CCGAGATTTT TGCCGAAGCC TGCCGCAAGG CGAAAGTCTG GGGCGTGTTC
150AM7_001   CCGAGATTTT CGCCGAGGCC TGCCGCAAGG CCAAGGTCTG GGGCGTGTTC
431am7_002   CTGCTGTGTT CGCCGACGCC TGCCGCAAGG CCAACGTATG GGGCGTGTTT
```

FIG. 7A

```
                                                          AAAG C
150am13_00  TCGCTCACCG GCGAACGTCA CGAGGAACAT CCGAAGAAGG CGCCCTACAA
150AM7_001  TCGCTGACCG GCGAGCGCCA CGAGGAGCAT CCCAATAAAG CGCCGTACAA
431am7_002  TCGCTGACGG GCGAGCGCCA CGAAGAGCAC CCGAACAAGG CGCCGTACAA
                                                       CAG AA
150am13_00  CACGCTGATC CTGATGAACG ACAAGGGCGA GGTGGTCCAG AAATACCGCA
150AM7_001  CACCCTGATC CTGATGAACG ACAAGGGTGA AGTCGTTCAG AAATATCGCA
431am7_002  CACGCTCATC CTGATGAACA ACAAGGGCGA GATCGTGCAG AAGTACCGCA
                                                    GGTA
150am13_00  AGATCATGCC GTGGGTTCCG ATCGAGGGCT GGTACCCCGG CAACTGCACC
150AM7_001  AGATCATGCC GTGGGTGCCG ATCGAAGGCT GGTATCCCGG CAACTGCACG
431am7_002  AGATCATGCC CTGGGTGCCG ATCGAAGGCT GGTATCCGGG CGATTGCACC
                                              TGAAG
150am13_00  TACGTCTCCG ACGGGCCGAA GGGCATGAAG GTTTCGCTGA TCATCTGCGA
150AM7_001  TACGTCTCCG AAGGCCCGAA GGGCATGAAG ATGTCGCTGA TCATCTGCGA
431am7_002  TATGTGTCGG AAGGCCCCAA GGGACTGAAG ATCAGCCTCA TCATCTGCGA
                                        TCTGGCG
150am13_00  TGACGGCAAC TATCCGGAAA TCTGGCGCGA CTGCGCCATG AAGGGCGCCG
150AM7_001  CGACGGCAAC TACCCGGAAA TCTGGCGTGA CTGCGCGATG AAGGGCGCCG
431am7_002  CGACGGCAAT TACCCCGAGA TCTGGCGCGA TTGCGCCATG CGCGGCGCCG
                                     CCAG
150am13_00  AGCTGATCGT GCGCTGCCAG GGCTACATGT ATCCGGCCAA GGACCAGCAG
150AM7_001  AACTGATCAT CCGCTGCCAG GGCTACATGT ATCCCGCCAA GGATCAGCAG
431am7_002  AGCTGATCGT GCGTTGCCAG GGATACATGT ACCCGGCCAA GGACCAGCAG
```

FIG. 7B

```
                                    GC
150am13_00  GTCATCATGG CGAAGGCGAT GGCGTGGGCG AATAATTGTT ACGTCGCGGT
150AM7_001  GTGCTGATGG CGAAAGCAAT GGCCTGGGCC AACAACGTTT ATGTCGCGGT
431am7_002  GTCATGGTGT CCAAGGCCAT GGCGTGGATG AACAACGTCT ACGTGGCGGT
                          GGGCTTCG
150am13_00  TTCCAATGCC GCGGGCTTCG ATGGCGTCTA TTCGTATTTC GGCCACTCGG
150AM7_001  CGCCAATGCC TCGGGCTTCG ACGGCGTCTA CTCGTATTTC GGCCATTCGG
431am7_002  GGCCAATGCC GCGGGCTTCG ACGGCGTGTA TTCCTACTTC GGCCATTCGG
                        TTCGA
150am13_00  CGATCATCGG CTTCGATGGC CGCACGCTCG GCGAATGCGG CGAGGAAGAA
150AM7_001  CGATCATCGG CTTCGACGGC CGTACCCTCG GCGAATGCGG CGAGGAGGAT
431am7_002  CCATCATCGG CTTCGACGGC CGCACGCTGG GCGAATGCGG TGAAGAAGAC
                      C AGTA
150am13_00  TACGGCATCC AGTATGCCCA GCTTTCGAAG ATGCTGATCC GCGACGCCCG
150AM7_001  TATGGCATCC AGTATGCCGC CATCTCCAAG TCGCTGATCC GCGACGCGCG
431am7_002  ATGGGCGTGC AGTACGCCGA GCTCTCCACC AGCCTGATCC GCGACGCGCG
                        CAATC
150am13_00  CCGCACCGGA CAATCGGAAA ACCATCTCTT CAAGCTGGTG CATCGTGGCT
150AM7_001  CCGCACCGGC CAATCGGAAA ACCATCTCTT CAAGCTGGTG CACCGTGGCT
431am7_002  CAAGAACATG CAGTCGCAGA ACCACTTGTT CAAGCTGGTG CACCGCGGCT
                        GATCAA
150am13_00  ACACCGGGTT GATCAACTCC GGCGAGGGCG ACCGCGGTCT CGCGGCCTGT
150AM7_001  ACACCGGCAT GATCAATTCC GGCGAGGGCG ACCGCGGTGT CGCGGCTTGC
431am7_002  ACACCGGCAA GATCAATTCC GGCGAAGAGG CCACCGGCGT CGCGGCATGC
```

FIG. 7C

```
                 TTA
150am13_00   CCTTATGAGT TCTACAACAA ATGGATCGCC GATCCGGAAG GCACCCGCGA
150AM7_001   CCGTATGATT TCTATTCGAA ATGGATCGCC GATCCCGAGG GTACACGCGA
431am7_002   CCGTACAACT TCTACGCCAA CTGGATCAAC GATCCGGAGG GCACGCGCAA
               ATGGT
150am13_00   AATGGTCGAG TCCTTTACCC GGCCGACGGT GGGAACCGAT GAAGCGCCCA
150AM7_001   GATGGTGGAA TCCTTCACGC GTCCGACGGT GGGTGTGGAG GAATGCCCGA
431am7_002   GATGGTCGAA TCCTTCACCC GGTCCACCGT GGGCACGCCG GAGTGCCCCA
             TCGAG
150am13_00   TCGAAGGCAT CCCGAACAAG GTCGCGGTGC ACCGCTGA   aagct
150AM7_001   TCGAGGGCAT TCCGAACAAG GCCACCACGC ACCGCTGA   aagct
431am7_002   TGGACGGCAT CCCCAACGAG GACGCCAAGC ACCGCTAG   aagct
                                                        HindIII
```

FIG. 7D

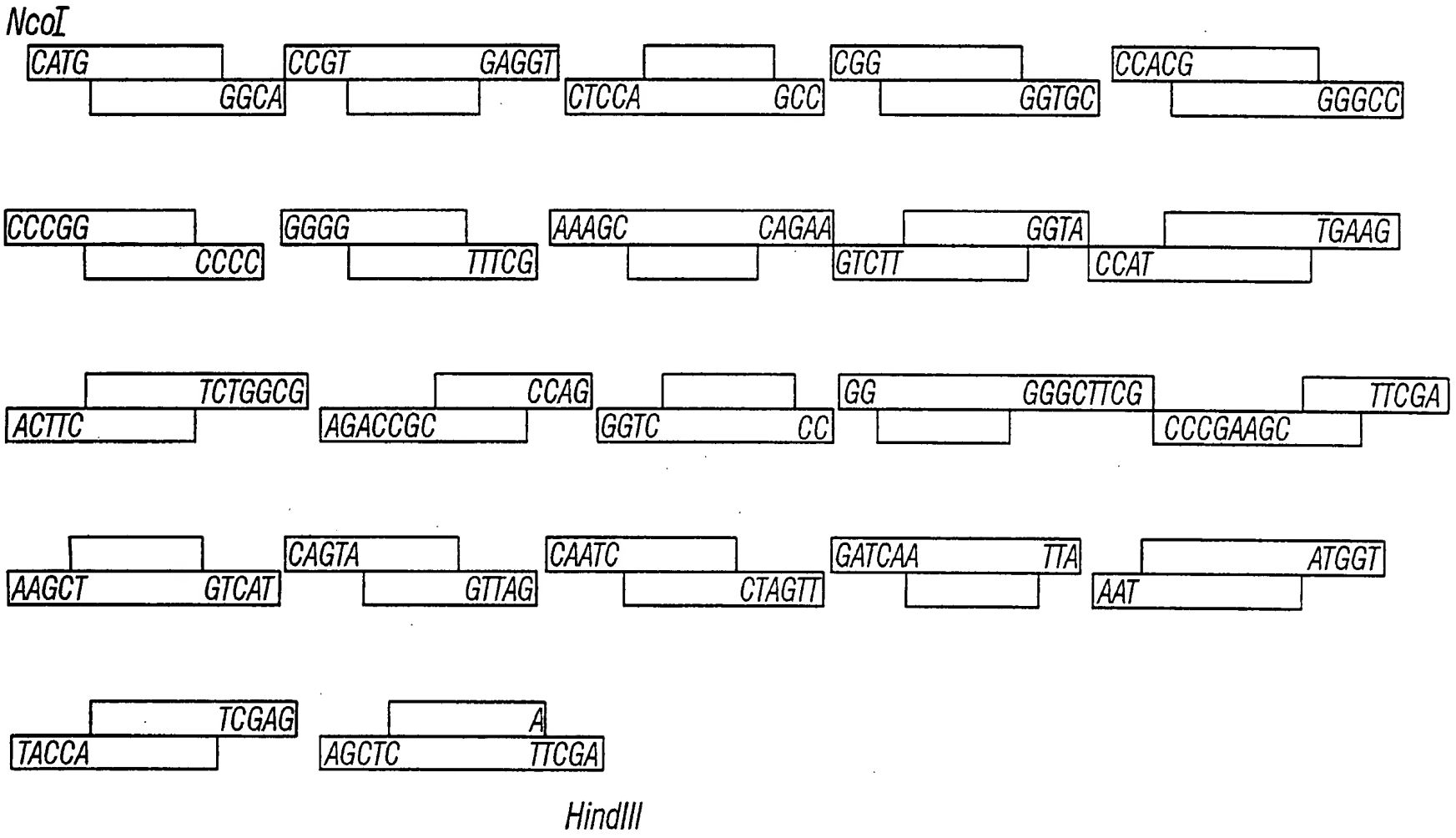

NcoI
CATG
GGCA
CCGT
GAGGT
CTCCA
GCC
CGG
GGTGC
CCACG
GGGCC
CCCGG
CCCC
GGGG
TTTCG
AAAGC
CAGAA
GTCTT
GGTA
CCAT
TGAAG
ACTTC
TCTGGCG
AGACCGC
CCAG
GGTC
CC
GG
GGGCTTCG
CCCGAAGC
TTCGA
AAGCT
GTCAT
CAGTA
GTTAG
CAATC
CTAGTT
GATCAA
TTA
AAT
ATGGT
TACCA
TCGAG
AGCTC
A
TTCGA
HindIII

FIG. 8

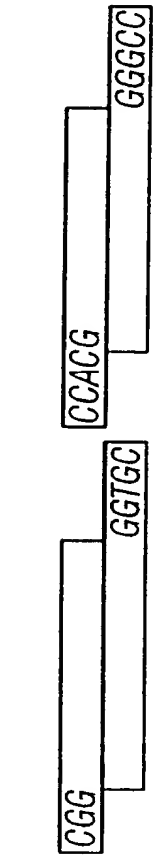
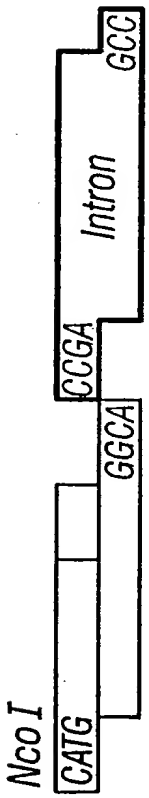

CCACG
GGGCC
CGG
GGTGC

FIG. 9


Nco I
CATG
GGCA
CCGA
Intron
GCC

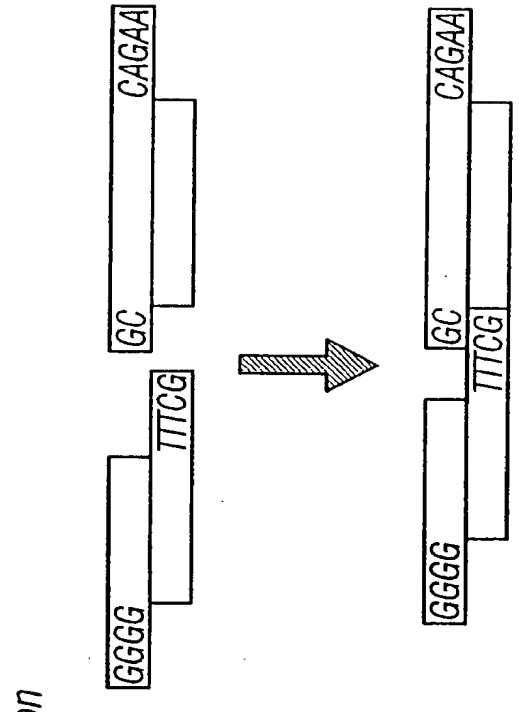

Gap Ligation
GGGG
TTTCG
GC
CAGAA
GGGG
TTTCG
GC
CAGAA

FIG. 10

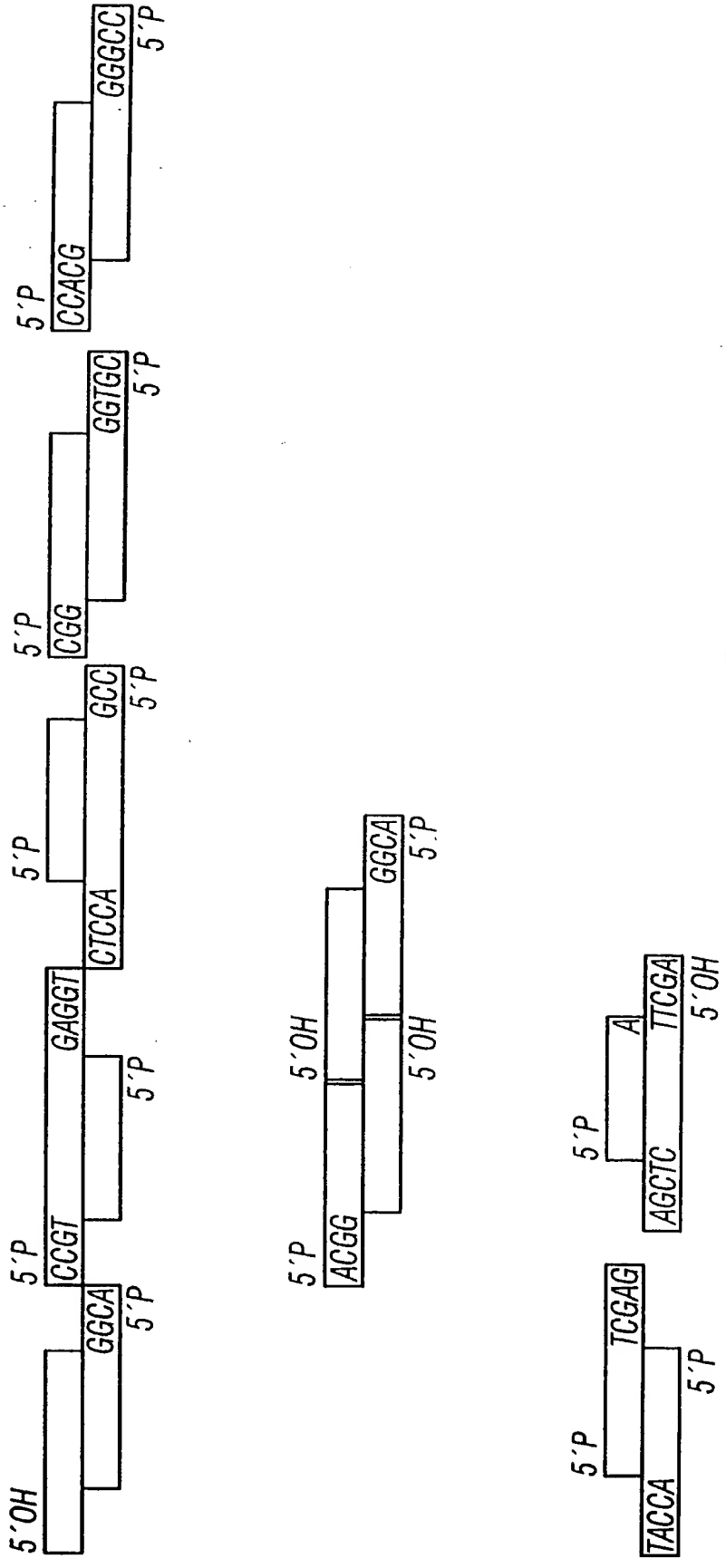

5′OH
GGCA
5′P
5′P
CCGT
GAGGT
5′P
5′P
CTCCA
GCC
5′P
5′P
CGG
GGTGC
5′P
5′P
CCACG
GGGCC
5′P
5′OH
ACGG
GGCA
5′P
5′OH
5′OH
5′P
AGCTC
TTCGA
5′OH
5′P
A
5′P
TACCA
TCGAG
5′P

FIG. 11

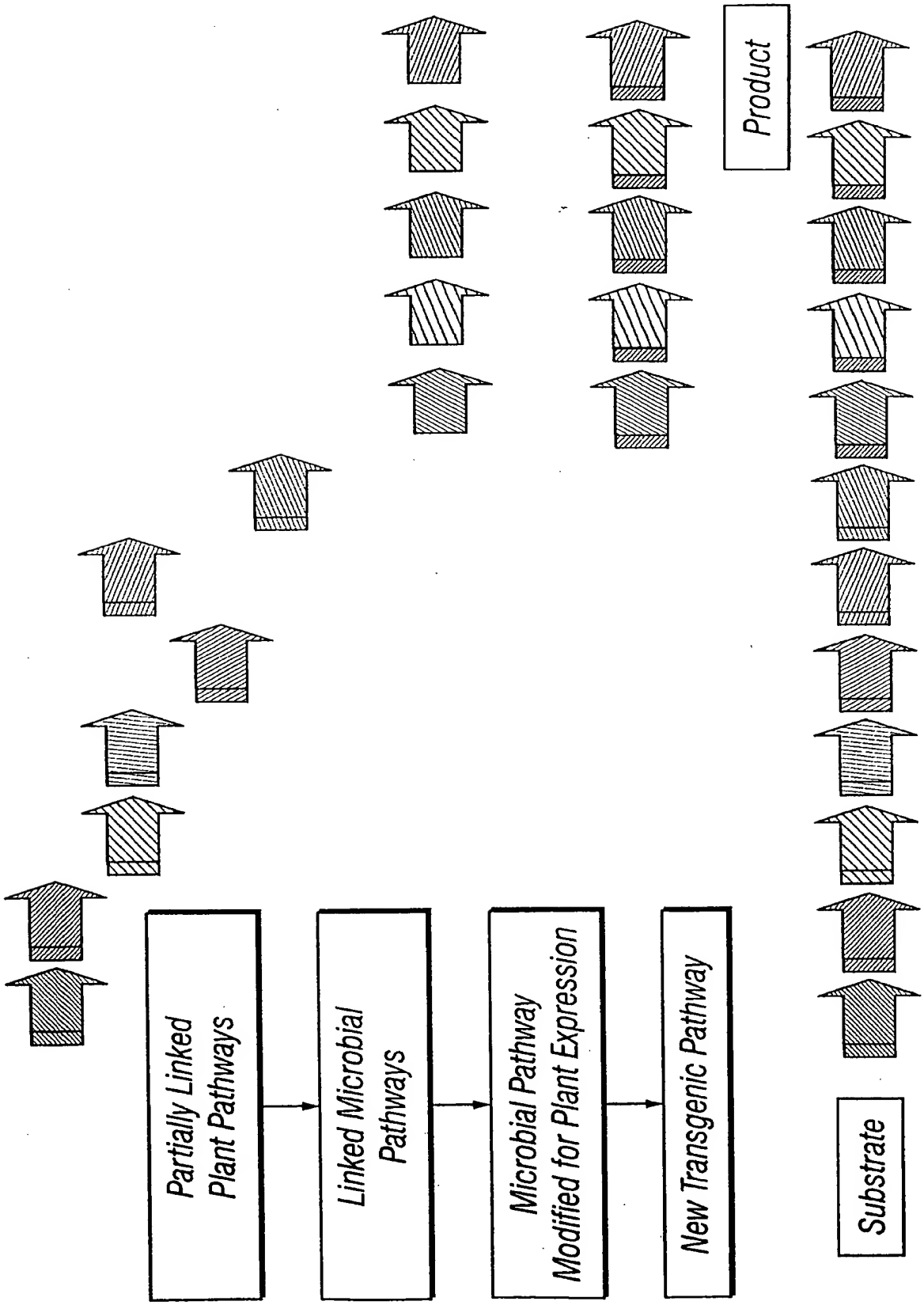

Partially Linked Plant Pathways
Linked Microbial Pathways
Microbial Pathway Modified for Plant Expression
New Transgenic Pathway
Substrate
Product

FIG. 12

BY ... SCIDLABS

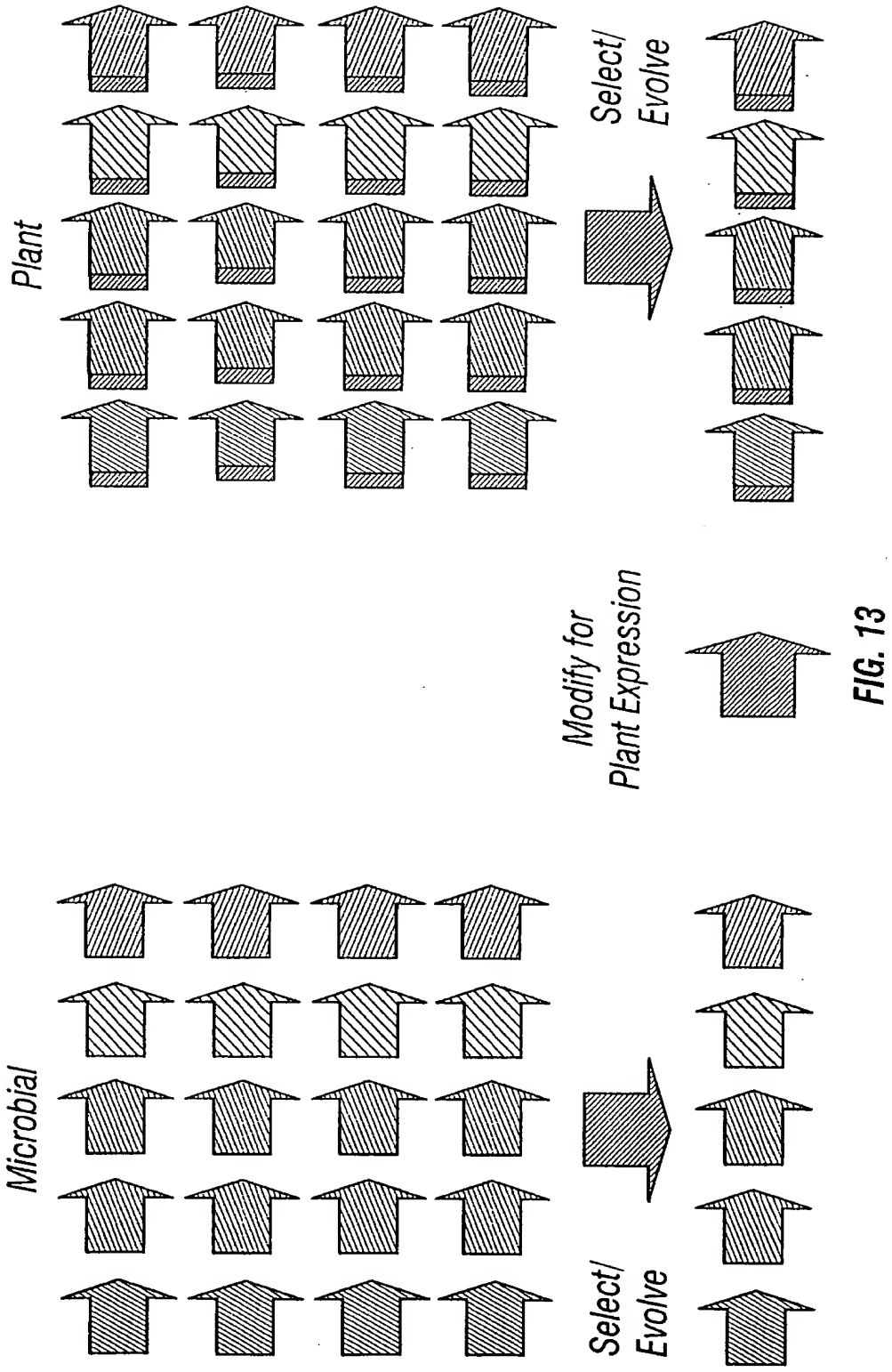


FIG. 13

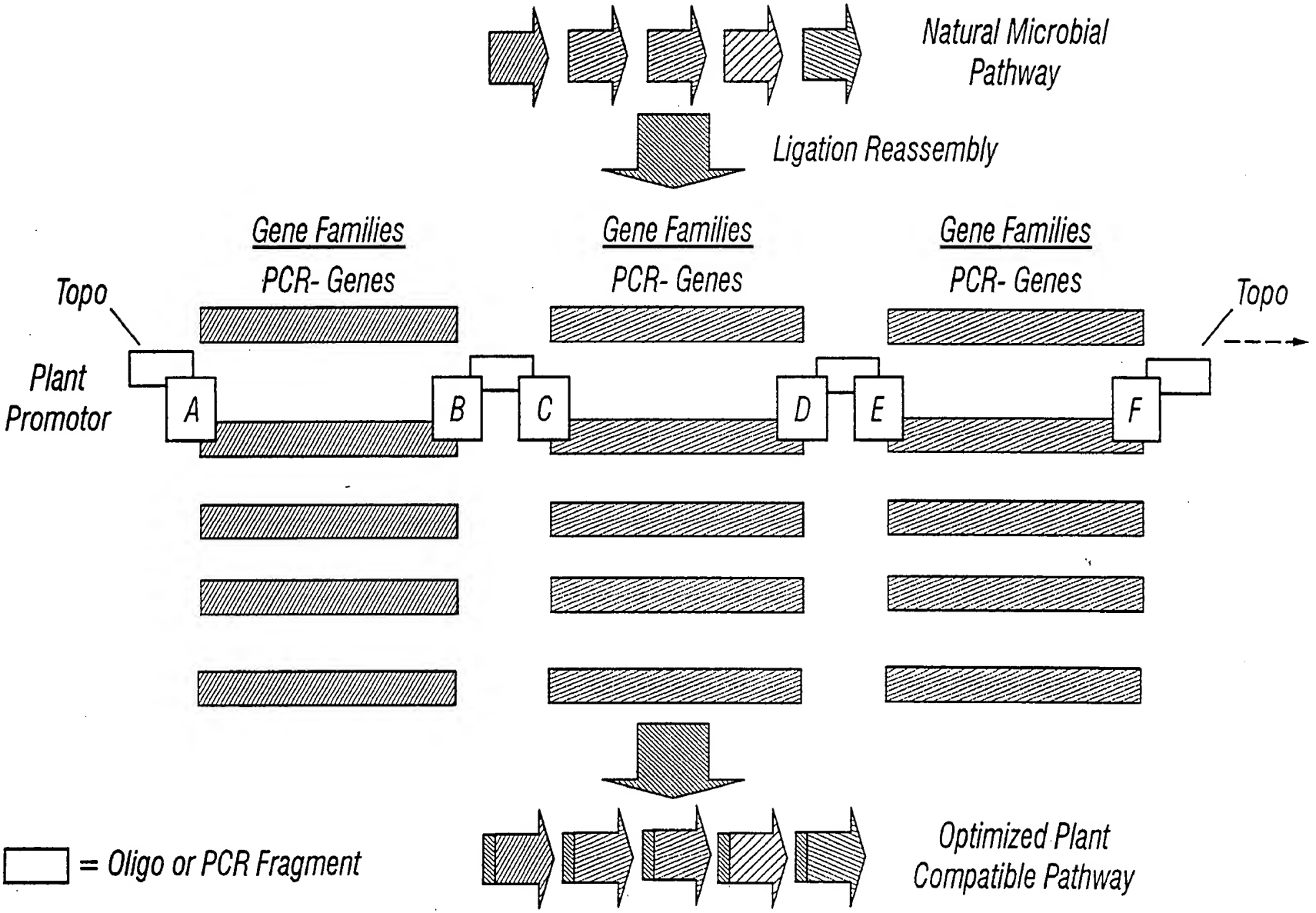
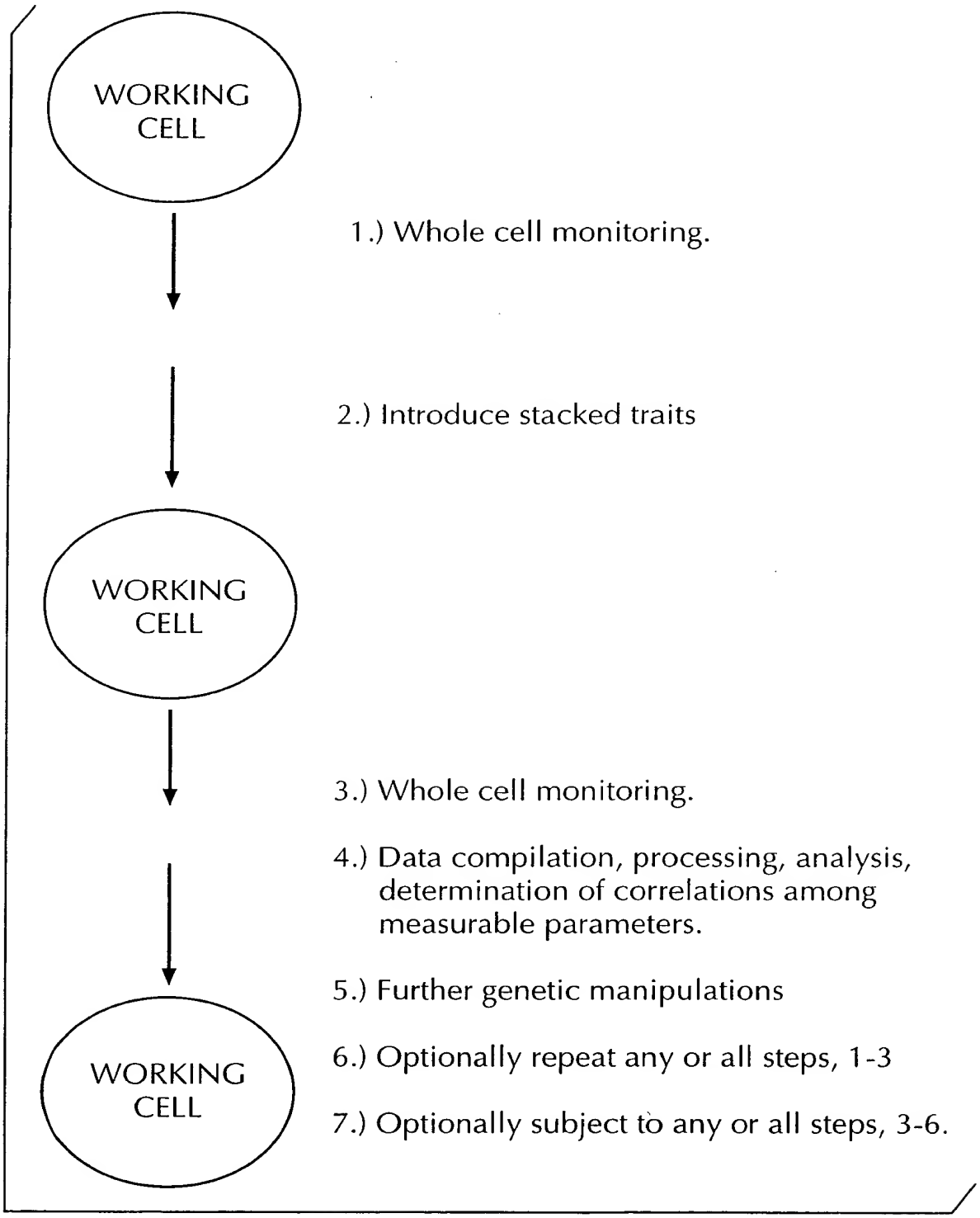

Natural Microbial Pathway
Ligation Reassembly
Gene Families
PCR- Genes
Gene Families
PCR- Genes
Gene Families
PCR- Genes
Topo
Topo
Plant Promotor
A
B
C
D
E
F
= Oligo or PCR Fragment
Optimized Plant Compatible Pathway

FIG. 14

FIG. 15


WORKING CELL
1.) Whole cell monitoring.
2.) Introduce stacked traits
WORKING CELL
3.) Whole cell monitoring.
4.) Data compilation, processing, analysis, determination of correlations among measurable parameters.
5.) Further genetic manipulations
WORKING CELL
6.) Optionally repeat any or all steps, 1-3
7.) Optionally subject to any or all steps, 3-6.

BY: SUBCLASS

FIG. 16

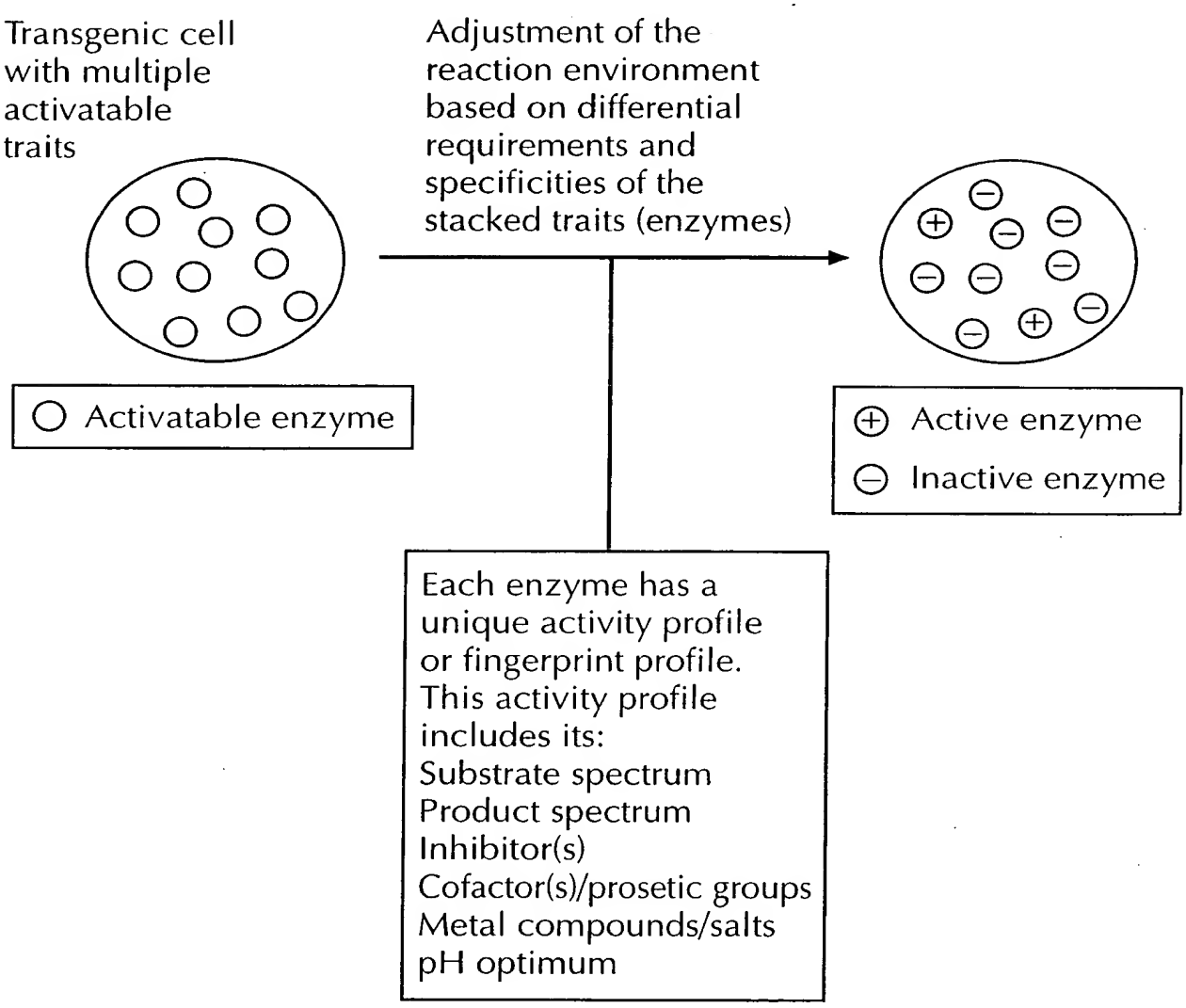


FIG. 17

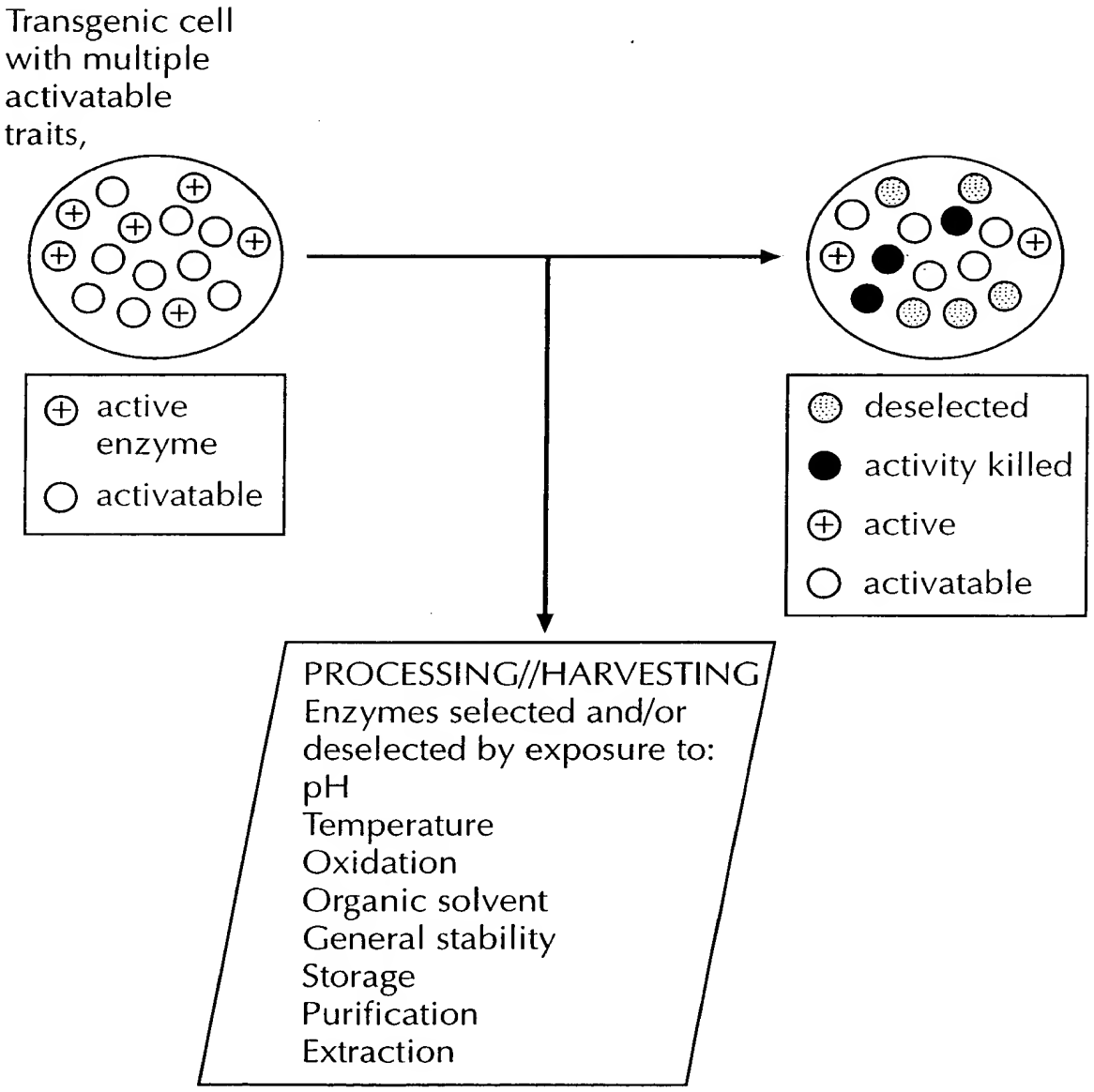

Transgenic cell with multiple activatable traits,
⊕ active enzyme
○ activatable
deselected
activity killed
⊕ active
○ activatable
PROCESSING//HARVESTING
Enzymes selected and/or deselected by exposure to:
pH
Temperature
Oxidation
Organic solvent
General stability
Storage
Purification
Extraction

FIG. 18

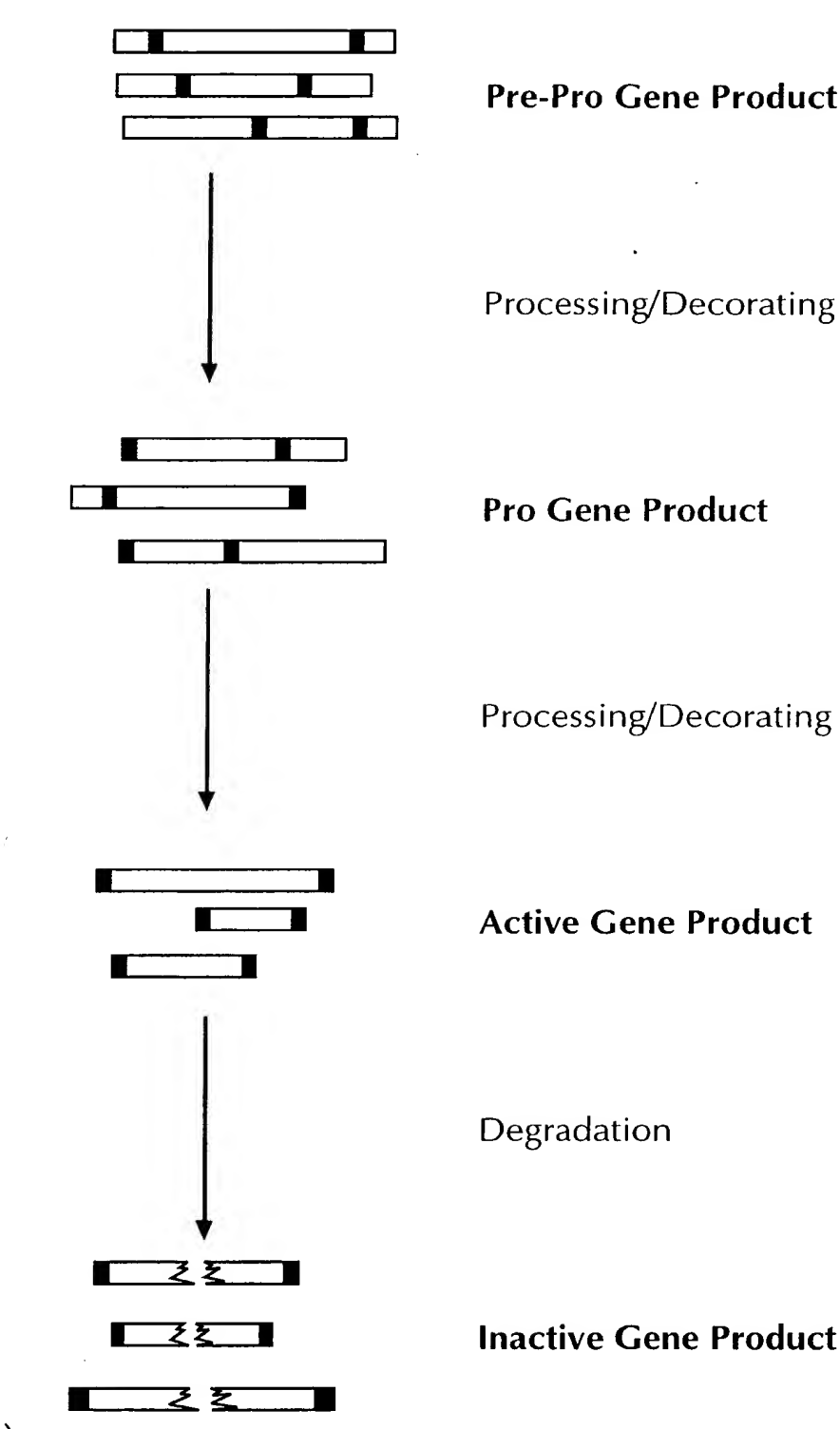

Pre-Pro Gene Product
Processing/Decorating
Pro Gene Product
Processing/Decorating
Active Gene Product
Degradation
Inactive Gene Product

FIG. 19

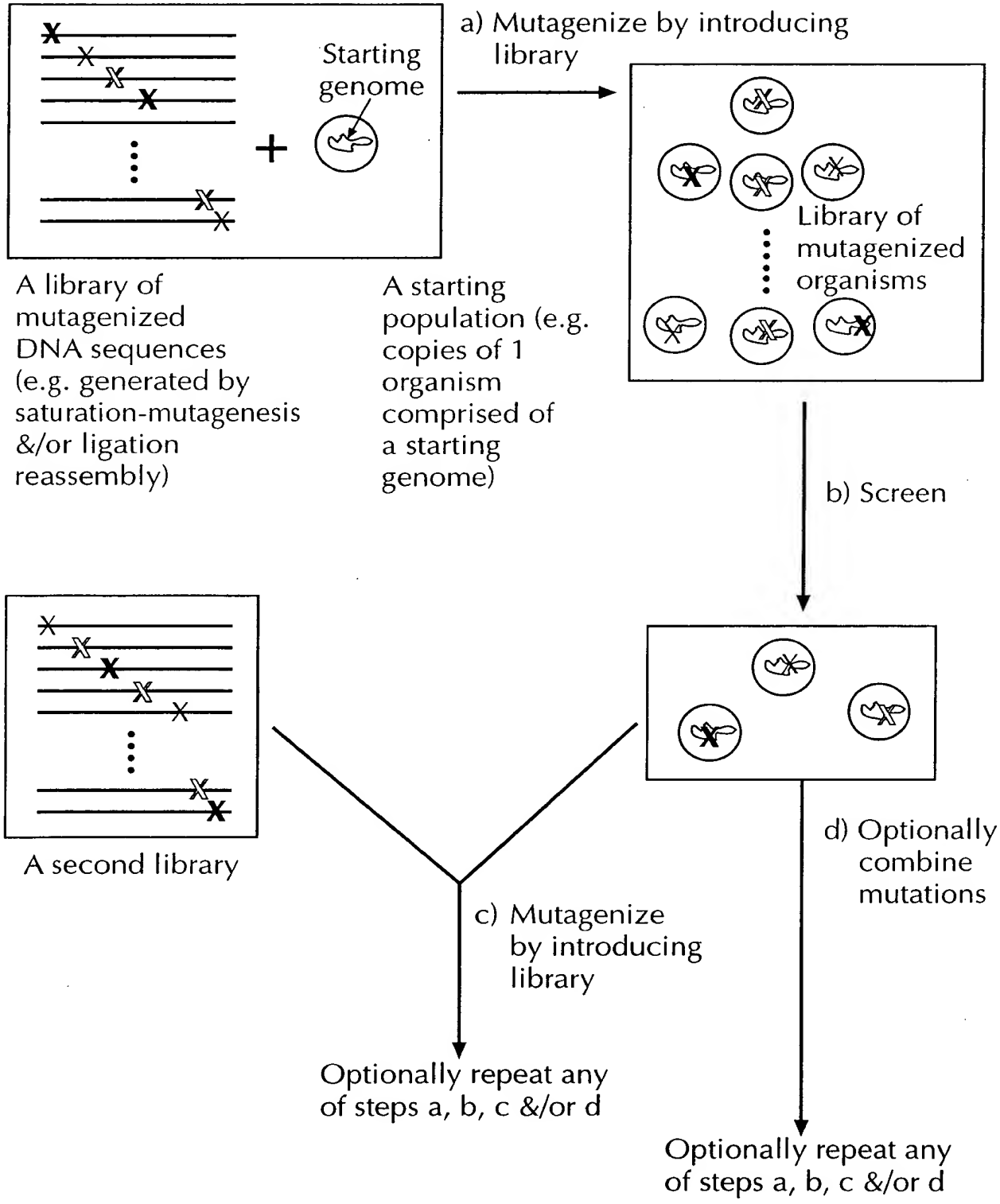

Starting genome
a) Mutagenize by introducing library
Library of mutagenized organisms
A library of mutagenized DNA sequences (e.g. generated by saturation-mutagenesis &/or ligation reassembly)
A starting population (e.g. copies of 1 organism comprised of a starting genome)
b) Screen
A second library
c) Mutagenize by introducing library
d) Optionally combine mutations
Optionally repeat any of steps a, b, c &/or d
Optionally repeat any of steps a, b, c &/or d

FIG. 20

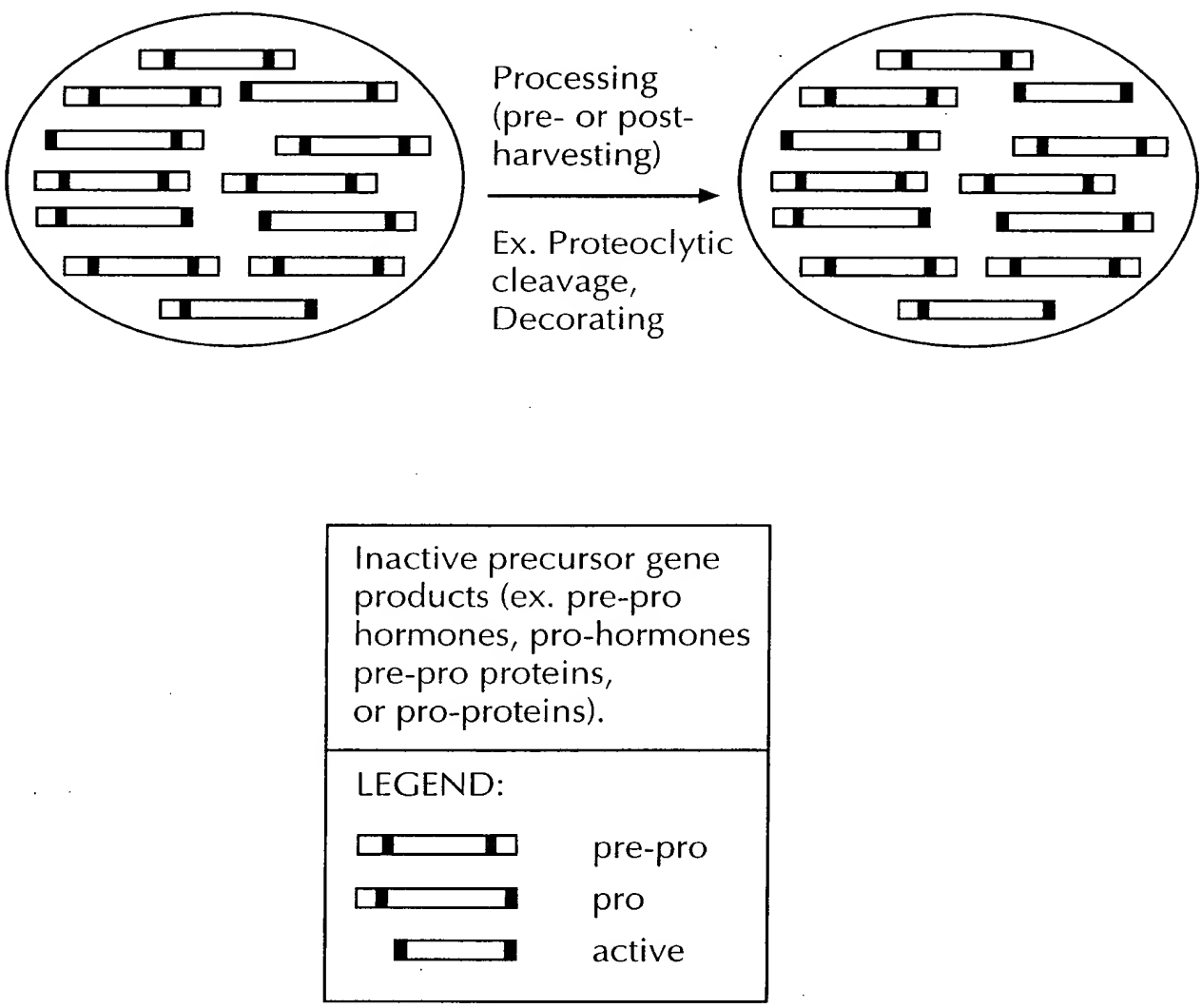

Processing
(pre- or post-
harvesting)
Ex. Proteoclytic
cleavage,
Decorating
Inactive precursor gene products (ex. pre-pro hormones, pro-hormones pre-pro proteins, or pro-proteins).
LEGEND:
pre-pro
pro
active

FIG. 21

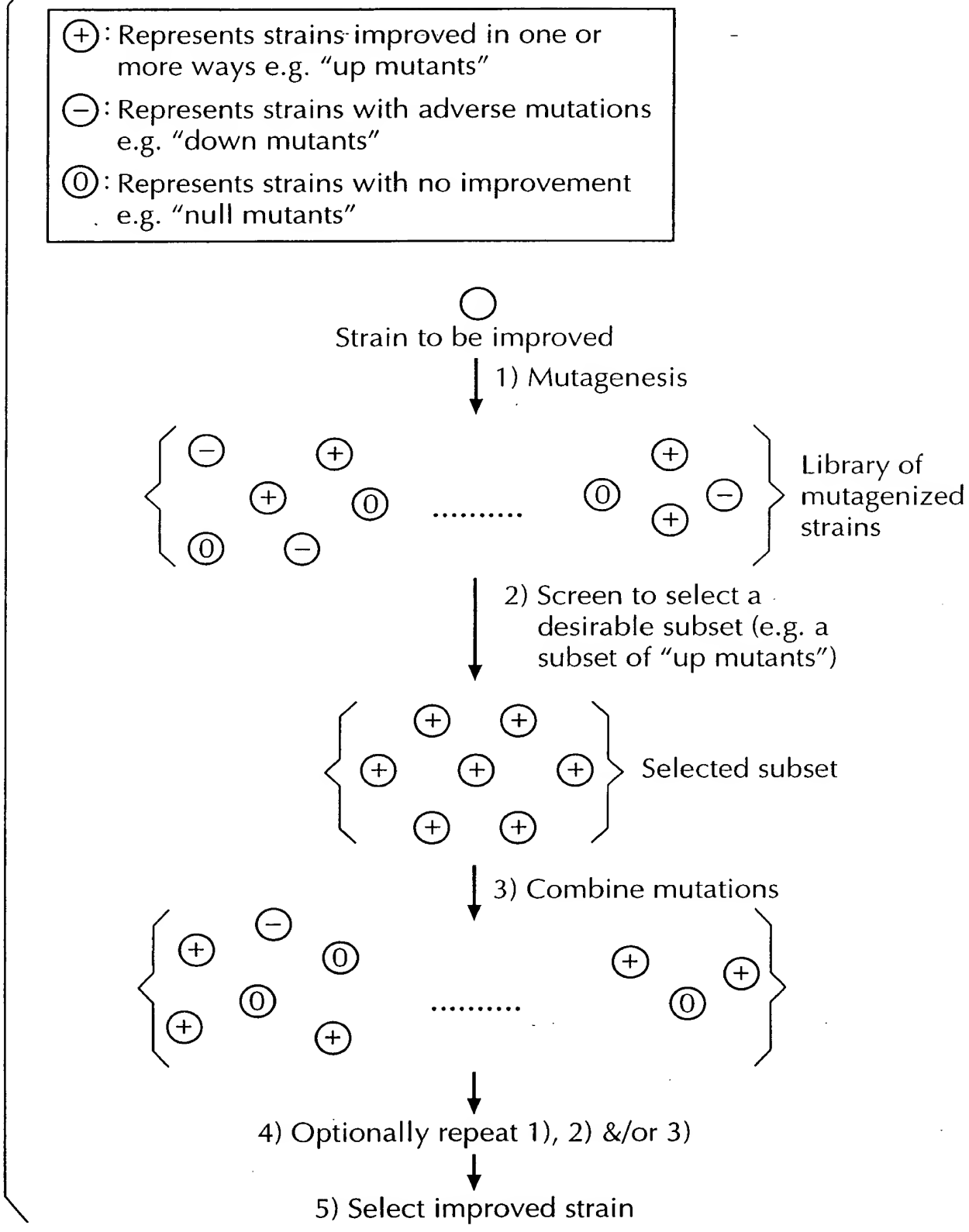

⊕: Represents strains improved in one or more ways e.g. "up mutants"
⊖: Represents strains with adverse mutations e.g. "down mutants"
⓪: Represents strains with no improvement e.g. "null mutants"
Strain to be improved
1) Mutagenesis
Library of mutagenized strains
2) Screen to select a desirable subset (e.g. a subset of "up mutants")
Selected subset
3) Combine mutations
4) Optionally repeat 1), 2) &/or 3)
5) Select improved strain

FIG. 22

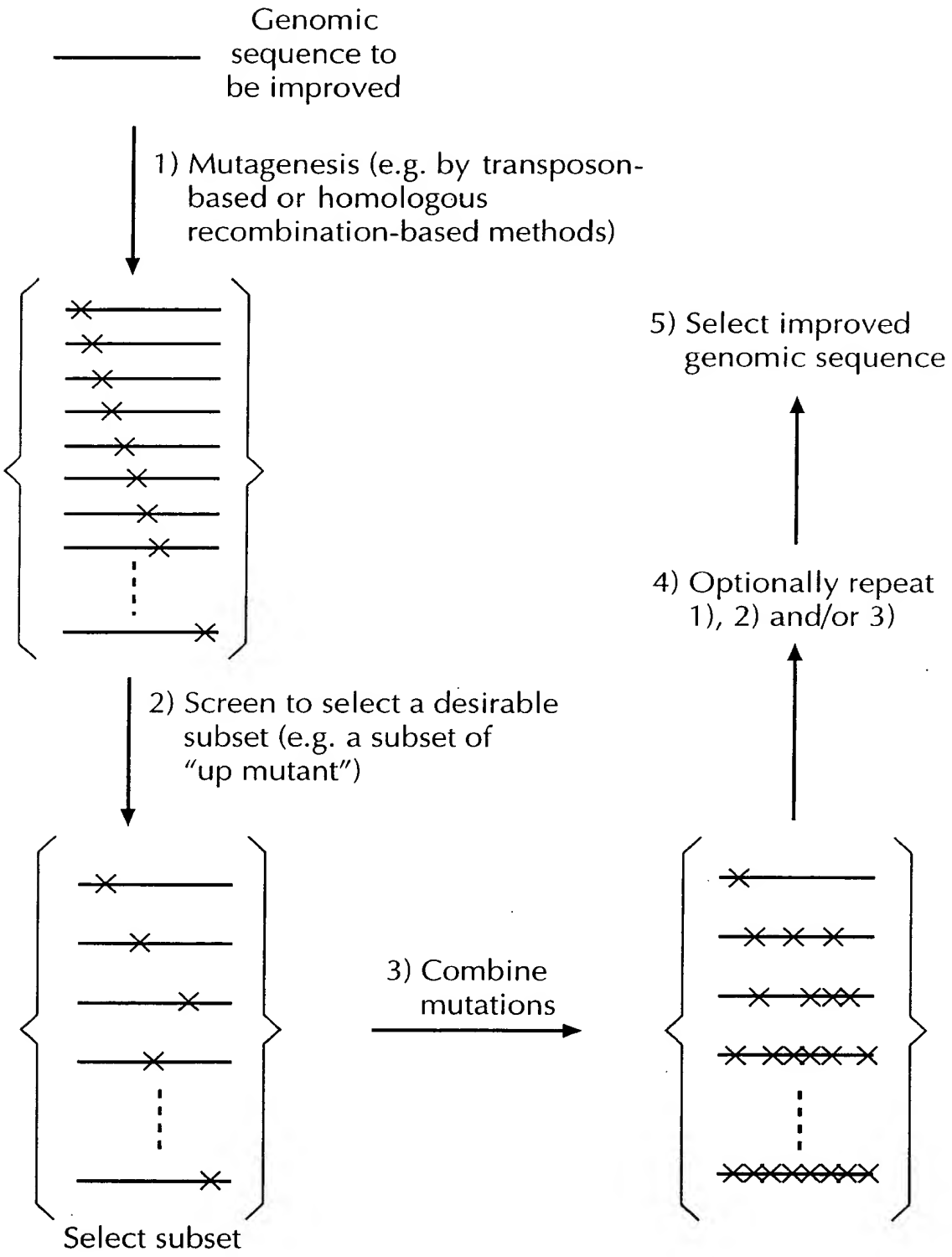
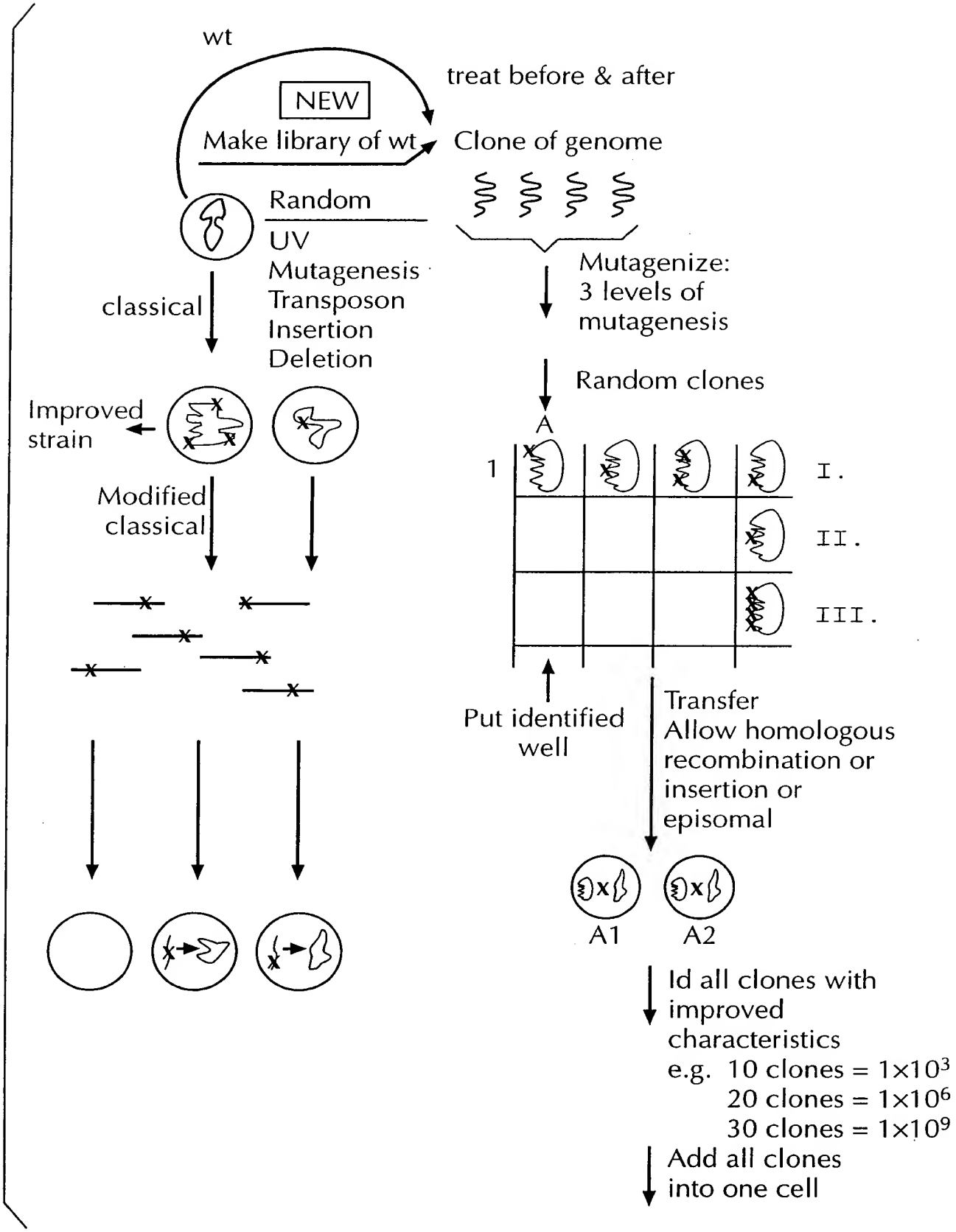

Genomic sequence to be improved
1) Mutagenesis (e.g. by transposon-based or homologous recombination-based methods)
2) Screen to select a desirable subset (e.g. a subset of "up mutant")
Select subset
3) Combine mutations
4) Optionally repeat 1), 2) and/or 3)
5) Select improved genomic sequence

FIG. 23


wt
NEW
Make library of wt
treat before & after
Clone of genome
Random
UV
Mutagenesis
Transposon
Insertion
Deletion
classical
Improved strain
Modified classical
Mutagenize: 3 levels of mutagenesis
Random clones
A
1
I.
II.
III.
Put identified well
Transfer
Allow homologous recombination or insertion or episomal
A1
A2
Id all clones with improved characteristics
e.g. 10 clones = 1×10³
20 clones = 1×10⁶
30 clones = 1×10⁹
Add all clones into one cell

APPROVED BY DRAFTSMAN CLASS SUBCLASS

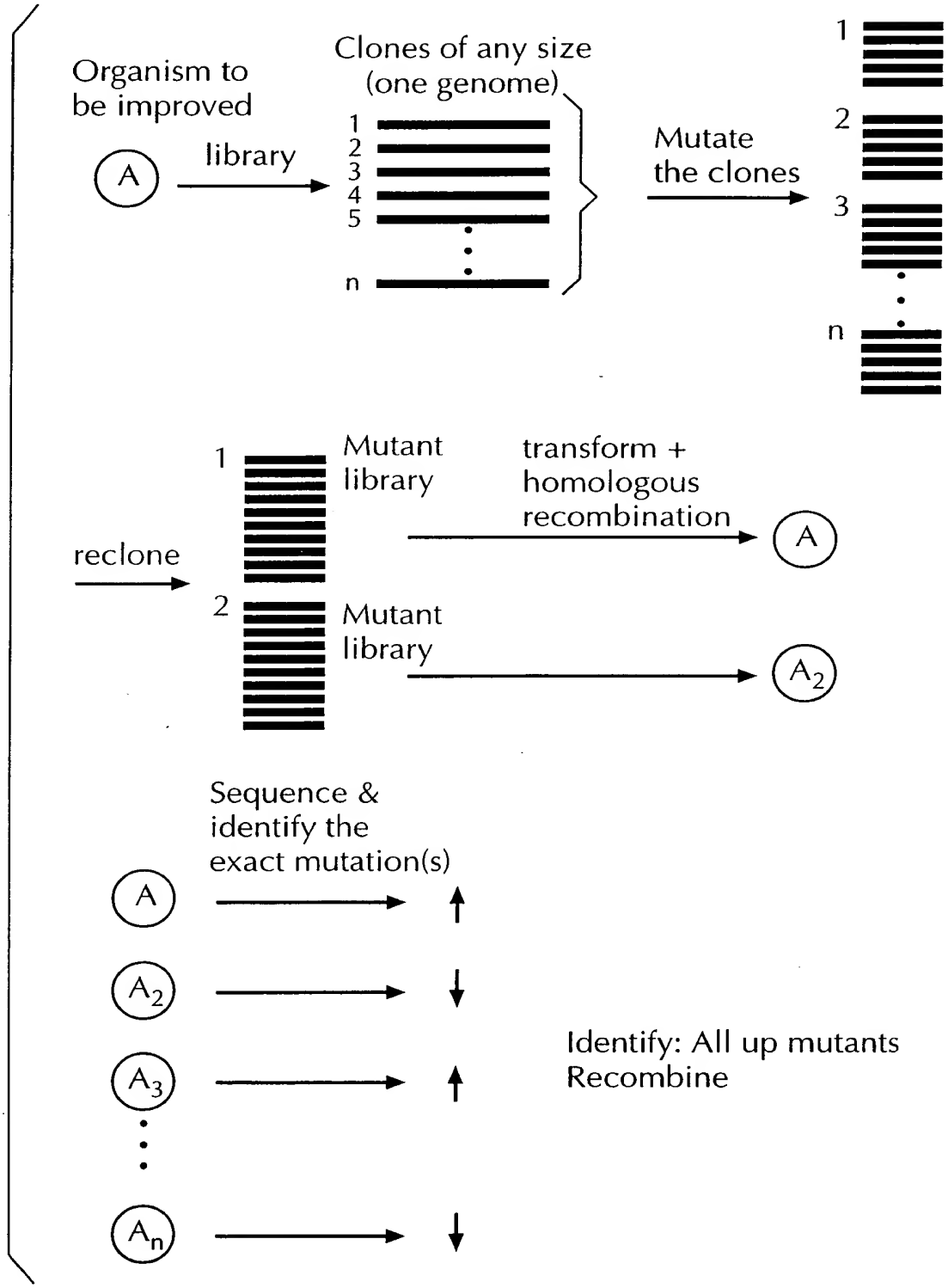



FIG. 24